

Listing of Claims

The following listing of claims will replace all prior versions, and listings, of claims in the subject application:

1. (currently amended) A method for reproducibly generating dendritic cells, comprising the steps of:

(a) obtaining blood mononuclear cells through ~~collection of~~ apheresis, with monocytes and monocyte precursors being separated substantially from lymphocytes, and loading the blood mononuclear cells into a cell culture container containing microcarrier beads and media for culturing dendritic cells therein, said media including ~~at least~~ rh-GM-CSF and at least one of rh-IL-4 or rh-IL-7 as a reagent reagents;

(b) incubating the contents of the cell culture container, including the media and the blood mononuclear cells loaded in the container in step (a), in order to grow dendritic cell culture;

(c) removing nonadherent cells which do not adhere to the beads after the incubation in step (b), from the cell culture container, by resuspending the contents of the cell culture container, allowing the microcarrier beads in the container to settle, and expressing off the supernatant out of the container; and

(d) further processing the ~~dendritic cell culture medium~~ contents of the cell culture container which remain in the container after step (c) and harvesting ~~the processed~~ dendritic cells from the contents.

2. (currently amended) A method for reproducibly generating dendritic cells, comprising the steps of:

(a) loading microcarrier beads and media for culturing dendritic cells into a cell culture container, said media

including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-7 as reagents;

(b) obtaining blood mononuclear cells through ~~collection of~~ apheresis, with monocytes and monocyte precursors being separated substantially from lymphocytes, and loading the blood mononuclear cells into the container;

(c) incubating the contents of the cell culture container, including the media and the mononuclear cells loaded in the container in step (b), in order to grow dendritic cell culture;

(d) removing from the cell culture container nonadherent cells which do not adhere to the beads after the incubation in step (b), by resuspending the contents of the cell culture container, allowing the microcarrier beads in the container to settle, and expressing off the supernatant out of the container; and

(e) further processing the ~~dendritic cell culture medium~~ contents of the cell culture container which remain in the container after step (d) and harvesting the ~~processed~~ dendritic cells from the contents.

3. (original) The method of claim 1, wherein the container comprises a gas permeable cell culture bag.

4. (original) The method of claim 1, wherein the container is a closed vessel.

5. (currently amended) The method of claim 1, wherein step (c) further includes washing the ~~tissue culture~~ blood mononuclear cells incubated in step (b) to remove nonadherent cells.

Claims 6 and 7 (canceled).

8. (currently amended) The method of claim 1, ~~wherein step (d)~~
includes further comprising:

(e) introducing additional media for culturing dendritic cells into the cell culture container after the nonadherent cells are removed from the cell culture container in step (b), said media including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-7;

(f) incubating the contents of the cell culture container after the additional media are introduced into the cell culture container in step (e); and

(g) agitating the contents of the container after the incubation in step (f).

9. (original) The method of claim 1, wherein after step (c) samples are removed from the container for quality control.

10. (original) The method of claim 9, wherein the quality control includes at least one of viability staining, microbial analysis, cell enumeration, microscopic examination of dendritic cell morphology, and immunophenotyping to determine a purity of the dendritic cell preparation.

11. (original) The method of claim 1, wherein the blood mononuclear cells are obtained by apheresis.

Claim 12 (canceled).

13. (currently amended) A method for reproducibly generating dendritic cells, comprising the steps of:

(a) obtaining blood mononuclear cells through ~~collection of apheresis~~, with monocytes and monocyte precursors being separated substantially from lymphocytes, and loading the blood mononuclear

cells along with medium for culturing dendritic cells into a cell culture container containing microcarrier beads therein, said medium including ~~at least~~ rh-GM-CSF and at least one of rh-IL-4 or rh-IL-7 as a reagent reagents;

(b) ~~preparing~~ incubating the contents of the cell culture container, including the medium and the blood mononuclear cells loaded into the cell culture container in step (a), for culturing, ~~by incubating said contents,~~ allowing beads with adherent cells attached thereto after incubation to settle, and then expressing off supernatant including nonadherent cells;

(c) introducing additional media for culturing dendritic cells into the cell culture container after the supernatant is expressed off in step (b), said additional media including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-7;

(d) incubating the contents of the cell culture container, ~~after the additional media are introduced into the cell culture container in~~ step (c), in order to grow dendritic cell culture; and

(e) harvesting dendritic cells from the dendritic cell culture in the container after step (d).